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Risk factors for fecal shedding of *Salmonella* in 91 US dairy herds in 1996

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Abstract

In 1996, data on management practices used on US dairy operations were collected and analyzed for association with fecal shedding of *Salmonella* by dairy cows. A total of 4299 fecal samples from 91 herds was cultured for *Salmonella* isolation. Herd-size (adjusted odds ratios (OR) = 5.8, 95% CI 1.1, 31.3), region (OR = 5.7, CI 1.4, 23.5), use of flush water systems (OR = 3.5, CI 0.9, 14.7), and feeding brewers' products to lactating cows (OR = 3.4, CI 0.9, 12.9) were identified as the most important predictive risk factors. The population attributable risks (PARs) for herd-size, region, flush water system, and feeding brewers' products to lactating cows were 0.76, 0.46, 0.37, and 0.42, respectively. The estimated PAR for all four risk factors combined was 0.95. The effects of these factors need to be more-closely evaluated in more-controlled studies, in order to develop intervention programs that reduce *Salmonella* shedding. © 2000 Elsevier Science B.V. All rights reserved.

Keywords: *Salmonella*; Shedding; Herd level; Cattle; Risk factors

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1. Introduction

Occurrence of *Salmonella* bacteria in food products poses health risks to consumers. This is reflected in the high cost of *Salmonella*-related illnesses in humans. In the US, the annual cost of non-typhoid foodborne salmonellosis in humans has been estimated at $0.6 billion to $3.5 billion (Buzby et al., 1996). This high cost is due to the high number of cases and the high impact of *Salmonella* within certain risk groups — making salmonellosis one of the most-costly bacterial human foodborne diseases (Buzby et al., 1996; Altekruse et al., 1997).

Dairy cattle are one source of *Salmonella* in food products, and several outbreaks of salmonellosis have been linked to beef and dairy products (Werner et al., 1979; Fierer, 1983; Davies et al., 1996). Unlike dairy products which are routinely pasteurized, beef from cull dairy cows (in addition to beef from cull beef cows and fed cattle) remains a potential source of *Salmonella* to humans. Dairy cattle are a major source of meat for hamburgers (Gay et al., 1994), and contribute about 25% of all non-fed beef available for consumption in the US (Smith et al., 1994; USDA–APHIS, 1996a). *Salmonella*, which is commonly isolated in nature, can also cause important production-related losses in cattle (Wray and Davies, 1996). Factors that have been postulated to increase the risk of *Salmonella* shedding by cattle include season of the year, feeding contaminated feeds to cattle, and improper manure management (Wray and Davies, 1996; Anderson et al., 1997; Kinde et al., 1996). Kinde et al. (1996) showed that *Salmonella* is ubiquitous in effluents from municipal sewage treatment plants — which results in contamination of water sources. New cattle introduced into the herd, rodents and birds with access to animal feeds, and other farm inputs including the environment may be sources of *Salmonella* on dairy cattle operations (Evans, 1996). The multiple potential sources suggest that reducing the risk of *Salmonella* infection and shedding will require an integrated approach.

Understanding the factors leading to human outbreaks of salmonellosis is essential for designing public-health educational programs and for improving food-processing procedures. The USDA–FSIS pathogen-reduction Hazard Analysis Critical Control Point (HACCP) system — a strategy based on knowledge of potential hazards (Smith and House, 1992; Cullor, 1995; Buntain, 1997) — has been put in place for meat and poultry slaughter and processing plants (USDA–FSIS, 1996). Another approach for potentially reducing human foodborne salmonellosis is to understand the factors which increase *Salmonella* shedding in food animals. Knowledge of risk factors (and subsequent reduction of *Salmonella* transmission on the farm) might decrease the risk of contamination throughout the rest of the food chain. However, on-farm critical control points for most pathogens (including *Salmonella*) are not well understood. Individual-cow studies suggest that feeding animal by-products, intercurrent diseases, and stress (transportation, overcrowding, etc.) may increase bovine salmonellosis, and also fecal shedding of *Salmonella* by cattle (House and Smith, 1997). In the US, information from large-scale studies of herd-level risk factors for *Salmonella* shedding by cattle is lacking. In one case-control study of dairy herds in California, feeding animal fat and obtaining animal feed from a particular feed source were identified as herd-level risk factors for *Salmonella* shedding (Anderson et al., 1997). Large herd-size, movement of animals subclinically infected with *Salmonella*, access of vermin to dairy feeds, free-stall housing, prolonged intensive feeding, and occurrence of enteric conditions (e.g., Johne's disease, bovine virus diarrhea, fascioliasis) are associated with salmonellosis and *Salmonella* shedding in cattle herds (Wray and Sojka, 1977; Bender, 1994; Smith and House, 1992; Fedorka-Cray et al., 1998; Wray and Davies, 1996; Evans, 1996).

This study was conducted with a sample of dairy herds from across the US (a) to evaluate postulated herd-level risk factors and other management practices for association with *Salmonella* shedding in dairy cows, and (b) to estimate the contribution of each of the identified risk factors to herd-level *Salmonella* shedding.

2. Materials and methods

2.1. Study design

In 1996, the National Animal Health Monitoring System (NAHMS) (a USDA — Animal and Plant Health Inspection Service (APHIS) program) initiated a national survey of dairy herds in the US (USDA–APHIS, 1996b). The purpose of the study was to provide information on management practices and health of US dairy cattle. This study was conducted from January to July, 1996. From a stratified sample of 1219 operations with at least 30 milking cows, a convenience sample of 100 herds from across the US was selected for participation in the fecal-culture stages of the study. Fifty herds with 30–99 milk cows (''small operations'') and 50 herds with 100 or more milk cows (''large operations'') were selected. Information on herd-size and composition, management practices, health, and biosecurity was collected using questionnaires by National Agricultural Statistics Service (NASS) enumerators, federal and state Veterinary Medical Officers, and Animal Health Technicians.

2.2. Sample collection and processing

Fecal specimens from 4299 milk cows in 91 herds were collected to detect *Salmonella* fecal shedding. No fecal samples were collected from nine of the 100 herds due to lack of participation by one state and difficulties in synchronizing producer availability and the quota of weekly samples that the laboratory could handle. Fecal samples were collected from both milk cows in lactation (''milking cows'') and other milk cows identified by the producer to be culled in the next seven days (''cull cows''). For fecal collection, small operations were visited once and a sample of not more than 40 cows (including all cull cows on the operation) was selected. Large herds were visited three times for fecal sample collection. On one visit, a sample of 50 milking cows and not more than 20 cull cows was selected. On each of the other two visits, scheduled with the herd manager to occur just before planned cow culling dates, all cull cows (if culls were $\leq$20) or a convenience sample of not more than 20 cull cows (if culls were >20) was selected. For both small and large operations, the sampling approach was designed to obtain a sample representative of all groups of cows on the operation. This sampling design allowed a 95% confidence of detecting at least one cow shedding *Salmonella*, if the herd prevalence was $\geq$5%. Repeat visits to increase the likelihood of obtaining fecal samples from cull cows (for another aspect of the study) were made only for large operations, due to the low number of cull cows on small operations.

A new palpation glove was used to collect a fecal sample from the rectum of each selected cow. Fecal samples were submitted to the USDA — Agricultural Research Service (ARS) — National Animal Disease Center (NADC), in Ames, Iowa, for *Salmonella* culture, and *Salmonella* isolates to National Veterinary Services Laboratory (NVSL) for serotyping. Results of *Salmonella* culture status, serogroups and serotypes of *Salmonella* recovered were then sent to the Centers for Epidemiology and Animal Health (CEAH), Fort Collins, Colorado where they were merged with data from questionnaires. Data processing and inferential analysis was performed at CEAH.

2.3. Data analysis

Questionnaire and laboratory data were validated and stored in a SAS data base (Statistical Analysis Systems Institute, Cary, NC). Univariable descriptive statistics on the data were obtained using the frequency procedure of SAS. Stratified analyses using Mantel–Haenszel procedures were also performed. The effect of different sampling strategies for small and large operations was evaluated using the Breslow–Day statistic for homogeneity of odds ratios over strata of variables while controlling for herd-size (Hosmer and Lemeshow, 1989; Stokes et al., 1996). The power to detect associations between independent variables and herd-level *Salmonella* shedding status was computed from the data obtained in this study using methods for cross-sectional studies described by Kelsey et al. (1986).

A herd was considered positive if there was at least one cow detected shedding any *Salmonella* serotype, and negative if no cow in the herd was detected shedding. Variables with a univariable chi-square p-value $\leq$0.2 were manually chosen from the list of variables studied and presented for further evaluation using multivariable procedures.

A multivariable logistic regression model (with herd positivity as the dependent variable and variables with a chi-square p-value $\leq$0.2 as independent variables) was fitted using a forward-selection procedure. A significance level of 0.1 was chosen for a variable to enter the model. Herd-size was automatically selected in the above model and was maintained in the refined model as a covariate to account for sampling differences between small and large herds. The significance of each variable to the model was assessed by its removal from the model and then comparing the deviances of the two hierarchical models using chi-square tables (Hosmer and Lemeshow, 1989). A variable was kept in the model if the p-value for the difference in deviance was <0.05. The general fitness of the model was determined using the Hosmer–Lemeshow statistic and by inspecting Pearson and deviance residual plots (SAS Institute, 1994). First-order interactions were assessed by creating dummy variables in the data step (Stokes et al., 1996). The interaction of herd-size with region and flush-water system could not be assessed because of low-frequency categories and presence of a zero cell. The relationships between variables were also assessed by cross-tabulating two variables and determining the chi-square value as a measure of association (an indicator of multicollinearity). The predictive accuracy of the fitted model was assessed using a receiver operating characteristic curve (SAS Institute, 1995). Due to only 91 herds in this study, it was not possible to exclude some herds from the analysis so that they can be used to test the predictive accuracy of the model. Thus, the ROC curve was generated from the same data used in the analysis.

The impact of modeled variables on the shedding of *Salmonella* in the sampled dairy cow population was estimated with the population attributable risk (PAR) using methods described by Bruzzi et al. (1985) and used in other studies (Wells et al., 1996; Evans, 1996). Adjusted odds ratios from multivariable logistic regression were used to estimate the relative risk (R_j) for each stratum (j) of each variable in the final model. Using ρ (the proportion of all herds with *Salmonella* shedders within stratum j), the PAR was determined as PAR $= 1 - \Sigma(\rho_j/R_j)$. The lower and upper 90% confidence interval (CI) of the odds ratio for each stratum (estimate of R_j) were used to estimate lower and upper bounds of PAR, respectively. The summary PAR for all the variables in the final model was determined using the above formula for PAR, except in this case odds ratios and proportion of herds shedding *Salmonella* were estimated from across all strata of the variables in the final model (Wells et al., 1996).

3. Results

3.1. Descriptive statistics of herds studied

Eighty seven of the 91 herds (95.6%) in the study had Holstein cows (Table 1). The mean percentage of Holstein cows on the operations was 91.0%. Sixty one herds had only Holstein cows, three only Jersey cows, and one only Ayrshire cows. There were no herds where Brown Swiss or Guernsey was the only breed on the operation. Fifty three percent of all herds fed lactating cows a total mixed ration (TMR). Most producers in the study (60.4%) participated in Dairy Herd Improvement Association programs. The median number of cows on the operation was 116 cows (range 36–2200). The median annual rolling herd average milk production per cow was 8433 kg with a range of 3882–11 818 kg. The attributes of herds in this study were compared (Table 2) with national estimates for US dairy operations with thirty or more cows (USDA–NASS, 1997).

3.2. Univariable analysis of postulated risk factors for Salmonella shedding

Salmonella fecal shedding was detected in 25 of the 91 herds (27.5%) surveyed. The number of herds with cows shedding *Salmonella* and the associated power of detecting a true association are presented in Table 3.

Management practices that were analyzed but did not reveal univariable associations with *Salmonella* shedding (at $p \leq 0.2$) included: access of animals (dogs, cats, birds, and rodents) to dairy feeds, methods of vermin control, water source (ponds, lakes, or stream), use of calving area as a sick pen, and water treatment by chlorination (Table 3). Frequency of cleaning water tanks, floor moisture in summer and winter, frequency of applying manure on pasture, feeding other supplements (corn silage, bakery products, clover, soybean meal), feeding in open feed bunks, number of days before cows are

Table 1
Description of herds ($n = 91$) studied for *Salmonella* risk factors

Variable	Levels	Number of herds
Breed composition as a percentage of the total number of cows on the operation	Herds with > 75% Holstein cows	82
	Herds with > 75% Jersey cows	4
	Herds with > 75% Ayrshire cows	1
	Herds with other or mixed breeds	4
Percentage of cows registered with a breed association	0	49
	1–49	21
	50–100	21
Annual rolling herd average milk production per cow (kg)	<6999	19
	7000–9999	56
	>10 000	16
Herd-size	30–99	42
	100–199	14
	200–499	17
	500–2999	18
Region[a]	Northwest	10
	Midwest	39
	Northeast	21
	Southeast	5
	Southwest	16

[a] Northwest (Oregon, Idaho, Washington), Midwest (Minnesota, Wisconsin, Illinois, Iowa, Missouri, Ohio, Indiana, Michigan), Northeast (New York, Pennsylvania, Vermont), Southeast (Tennessee, Florida), Southwest (California, New Mexico, Texas).

allowed on manured pastures, and methods of manure treatment also did not reveal univariable associations even at $p \leq 0.2$ (data not shown).

Herd-size and region may be proxies for other management practices, so the relationships of herd-size and region with other independent variables was assessed to better understand such relationships. Variables which showed remarkable relationships (as showed by chi-square p-value) with herd-size and region are given in Table 4.

3.3. Multivariable analysis

The six-variable model (season, region, herd-size, use of flush water system for manure handling, feeding lactating cows alfalfa, and feeding lactating cows brewer's products) obtained from the forward selection procedure was further refined by examining standard errors of all coefficients, chi-square p-values, and comparing models with and without season and alfalfa. The latter two variables were removed from the model, resulting in a better-fitting model (Table 5). Season and region were correlated ($\chi = 27.3$, df $= 1$), and based on model coefficients and standard errors of covariates in models with and without region or season, region was selected for the final model. The final model is given below.

Table 2
Comparison of operations studied for *Salmonella* shedding with US dairy operations with thirty or more cows (USDA–APHIS, 1996b; NASS cattle report, USDA–NASS, 1997)

Variable	Levels	US estimate (herds with ≥ 30 cows)	Estimate from study herds
Average annual milk production per cow (kg)		7540	8370
Percent of operations with Holsteins as the main breed (%)		93	90
Use DHIA programs (%)		43	60
Distribution of operations by region (% of the total)	Northwest	3	11
	Midwest	53	43
	Northeast	21	23
	Southeast	2	6
	Southwest	5	18
Distribution of operations by herd-size (% of the total)	30–99	75	46
	100–199	17	15
	≥ 200	8	39

There were no significant interactions detected. The interaction between farm size and region could not be estimated because there were no positive small operations in the south. To further evaluate key factors, the Breslow–Day test for homogeneity of odds ratios across strata was used. The effects of region and feeding brewers' products (as judged from univariable odds ratios) were not different in small and large operations as shown by Breslow–Day chi-square *p*-values of 0.43 and 0.11, respectively. There were no small operations which used flush water systems, thus this statistic could not be computed because of complete separation in the data (Hosmer and Lemeshow, 1989).

Herd has *Salmonella* shedders $= -3.73 + 1.74$ (region) $+ 1.76$ (herd-size) $+ 1.26$ (manure removal by flushing with water) $+ 1.21$ (feeding brewer's products).

The model appeared to fit the observed data well as shown by the Hosmer–Lemeshow statistic of 2.03 (4 df, $p = 0.73$) and the deviance statistic of 4.06 (6 df, $p = 0.67$). Inspection of Pearson and deviance residual plots did not reveal lack of fit in the model.

The model-predicted probability of a herd being positive given various possible unique combinations of levels of risk factors in the final model is provided in Table 6.

The estimates of PAR, confidence limits of PAR for individual modeled variables, and a summary PAR for all variables are in Table 7.

The receiver operating characteristic (ROC) curve of the multivariable logistic regression model is shown in Fig. 1. The ability of the model to discriminate between herds with *Salmonella* shedders and those without was satisfactory (C-statistic, 0.878). The curve rises quickly in the range where false positives are <0.15, indicating good predictive ability. However, this ROC curve was generated from the same data set as that used in the analysis. Thus there is a need to test this model on an independent data set.

Because there were a number of variables which were related to herd-size and region (and may have been hidden within the logistic model by herd-size and region), a second

Table 3
Percentage of herds with *Salmonella* shedders distributed by variables hypothesized to be risk factors for *Salmonella* shedding on US dairy operations

Description of the variable	Level of variable	Number of herds	Percent with *Salmonella* shedders	Power to detect a true association (%)
Herd-size (number of milk cows on the operation)[a]	≥100	49	46.9	99.4
	≤99	42	4.8	
Region of the country[a,b]	South	21	66.7	99.6
	North	70	15.7	
Season when most cows in the herd were sampled[a]	Summer	34	38.2	42.9
	Spring	57	21.1	
New cattle introduced to the herd in the past year[a]	Yes	52	32.7	25.1
	No	39	20.5	
Use of *Salmonella* vaccine[a]	Yes	21	42.9	57.9
	No	69	23.2	
Annual average milk production per cow (kg)[a]	≥ 8000	59	32.2	25.1
	< 8000	31	19.4	
Manure disposal by irrigation[a]	Yes	20	45.0	65.2
	No	68	19.1	
Manure disposal by broadcasting on pastures[a]	Yes	78	21.8	49.2
	No	10	50.0	
Manure treated in an uncovered anaerobic lagoon[a]	Yes	30	50.0	92.2
	No	61	16.4	
Manure equipment also used for handling heifer feeds[a]	Weekly	12	50.0	46.4
	Rarely or never	79	24.1	
Use of flush water system to remove manure from alleys[a]	Yes	19	68.4	99.4
	No	72	16.7	
Recycle[a] flush water	Yes	14	71.4	NA
	No	5	60.0	

	No flush system	72	16.7	
Use gutter cleaners to remove manure from alleys[a]	Yes	32	9.4	81.3
	No	59	37.3	
Use alley scrapers to remove manure from alleys	Yes	63	27.0	96.4
	No	28	28.6	
Chlorinated drinking water provided	Yes	13	23.1	5.7
	No	78	28.2	
Automatic waterer for use by individual cow provided[a]	Yes	32	9.4	81.3
	No	59	37.3	
Use of shared automatic waterers for cows[a]	Yes	38	18.4	37.5
	No	53	34.0	
Use of communal water tanks for cows[a]	Yes	75	32.0	54.8
	No	16	6.3	
Lactating cows fed a total mixed ration[a]	Yes	48	39.6	78.2
	No	43	14.0	
Lactating cows fed alfalfa[a]	Yes	62	21.0	52.8
	No	29	41.4	
Lactating cows fed cotton seeds or hulls[a]	Yes	50	40.0	84.1
	No	41	12.2	
Lactating cows fed cotton-seed meal[a]	Yes	14	57.1	77.0
	No	77	22.1	
Lactating cows fed at least one type of cotton meal[a]	Yes	55	41.8	25.1
	No	36	5.6	
Lactating cows fed brewer's products[a]	Yes	43	34.5	32.3
	No	48	20.8	
Lactating cows fed meat or bone meal	Yes	31	35.5	23.6
	No	60	23.3	
Lactating cows fed tallow	Yes	24	33.3	10.6
	No	66	25.8	

Table 3 (*Continued*)

Description of the variable	Level of variable	Number of herds	Percent with *Salmonella* shedders	Power to detect a true association (%)
Feed storage prevents access to dogs	Yes	49	28.6	4.3
	No	42	26.2	
Feed storage prevents access to cats	Yes	48	29.2	5.8
	No	43	25.6	
Feed storage prevents access to birds	Yes	49	28.6	4.4
	No	42	26.2	
Feed storage prevents access to rodents	Yes	42	31.0	10.2
	No	49	24.5	
Use chemicals to control rodents	Yes	53	26.4	2.9
	No	38	29.0	
Use traps to control rodents	Yes	10	30.0	3.8
	No	81	27.2	
Use cats to control rodents	Yes	82	25.6	77.6
	No	9	44.4	
Calving area used as a hospital area	Yes	47	23.4	32.6
	No	42	33.3	

[a] $p \leq 0.2$; offered to the multivariable model.

[b] South (California, New Mexico, Texas, Florida, and Tennessee), North (Oregon, Washington, Idaho, Minnesota, Illinois, Indiana, Iowa, Michigan, Wisconsin, Missouri, Ohio, New York, Vermont, and Pennsylvania).

Table 4
Relationship of herd-size and region with other variables evaluated for Salmonella shedding

Variable	Levels	No. of herds (Herd-size)		P^a	No. of herds (Region)		P^a
		Small	Large		North	South	
Lactating cows fed cotton seed products	Yes	0	38	0.001	31	19	0
	No	30	11		39	2	
Lactating cows fed brewers' products	Yes	16	27	0.110	35	8	0.34
	No	26	22		35	13	
Manure treated in uncovered lagoons	Yes	2	28	<0.001	16	14	0.001
	No	40	21		54	7	
Manure removed from alleys with flush water	Yes	0	19	<0.001	7	12	0.001
	No	42	30		63	9	
Manure removed from alleys with gutter cleaners	Yes	26	6	0.001	32	0	<0.001
	No	16	43		38	21	
Automatic waterer for use by individual cow provided	Yes	24	8	0.001	30	2	<0.001
	No	18	41		40	19	
Season of sampling	Spring	35	22	0.001	54	3	<0.001
	Summer	7	27		16	18	

[a] In cells where the frequency counts (number of herds) are <5, the *p*-values given are from the Fisher's exact test; others are from the chi-square test.

model removing herd-size and region was fit to further evaluate the relationship of these other variables (e.g., use of flush water, use of manure equipment for handling feeds, feeding lactating cows brewer's products, alfalfa, cotton-seed meal, and feeding a total mixed ration) with *Salmonella* shedding. A model obtained from the above variables using a less-conservative approach (significance level for entry into the model = 0.2) was used to identify hypotheses that need further evaluation. Herd-level risk factors identified from the second model included: use of flush water, use of manure equipment for handling feeds, feeding lactating cows brewer's products , alfalfa, cotton seed meal, and feeding a total mixed ration (Table 8).

Table 5
Final multivariable model of risk factors associated with *Salmonella* shedding in US dairy herds

Variable	*b*	se(b)	*P*	Odds ratio (OR)	95% CI of OR
Region	1.74	0.72	0.02	5.7	1.4, 23.5
Herd-size	1.76	0.86	0.04	5.8	1.1, 31.3
Flush-water system	1.26	0.73	0.08	3.5	0.9, 14.7
Brewer's products	1.21	0.67	0.08	3.4	0.9, 12.9

Table 6
Probability of detecting a herd with at least one cow shedding *Salmonella*

Use a flush-water system for manure removal	Feed brewers's products to lactating cattle	Herd-size (number of milk cows)	Region of the country	Predicted probability of *Salmonella* shedding	95% CI (probability)
No	No	≤ 99	North	0.023	0.01, 0.11
No	Yes	≤ 99	North	0.075	0.02, 0.27
No	No	≤ 99	South	0.121	0.02, 0.48
No	No	≥ 100	North	0.122	0.03, 0.38
No	Yes	≥ 100	North	0.319	0.16, 0.54
Yes	No	≥ 100	North	0.330	0.10, 0.68
No	No	≥ 100	South	0.443	0.16, 0.77
Yes	Yes	≥ 100	North	0.623	0.29, 0.87
No	Yes	≥ 100	South	0.728	0.38, 0.92
Yes	No	≥ 100	South	0.738	0.45, 0.91
Yes	Yes	≥ 100	South	0.904	0.65, 0.98

Table 7
Population attributable risks for factors associated with shedding of *Salmonella* on US dairy operations

Variable	PAR	CI of PAR
Use of flush water system for manure removal	0.37	0.04, 0.48
Feeding brewer's products to lactating cattle	0.42	0.05, 0.54
Herd-size	0.76	0.27, 0.88
Region	0.46	0.24, 0.53
All variables combined	0.95	NA

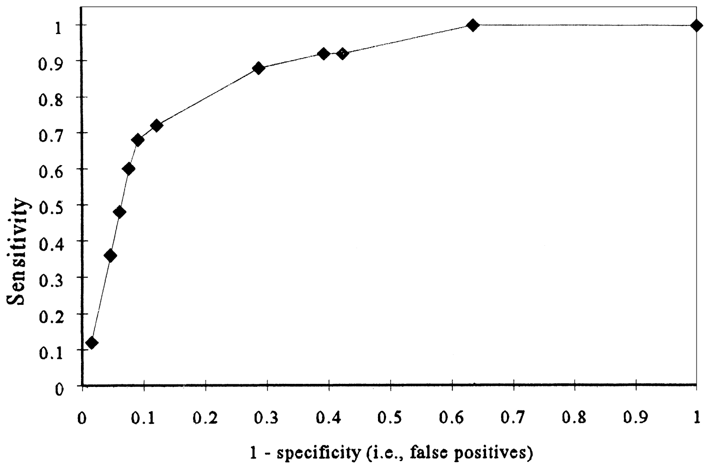

Fig. 1. Receiver operating characteristic curve showing the predictive accuracy of the logistic regression model for *Salmonella* shedding in dairy cattle.

Table 8
A multivariable model of risk factors associated with *Salmonella* shedding in US dairy herds obtained when herd-size and region were not offered to the model

Variable	*b*	se(b)	*P*	Odds ratio (OR)	95% CI of OR
Use a flush-water system	2.18	0.71	<0.01	8.9	2.2, 35.6
Feeding brewers' products to lactating cows	0.86	0.64	0.18	2.4	0.68, 8.2
Feeding alfalfa to lactating cows	−0.79	0.66	0.23	0.5	0.1, 1.7
Feeding cotton seed to lactating cows	1.84	0.74	0.01	6.3	1.5, 26.8
Feeding total mixed ration to lactating cows	1.10	0.69	0.11	3.0	0.8, 11.6
Manure equipment also used for handling feeds	1.18	0.80	0.14	3.2	0.7, 11.6

4. Discussion

Herds surveyed for *Salmonella* shedding in this study were similar to the US dairy cow population with respect to regional distribution and breed composition, but were somewhat larger, had higher milk production, used DHIA programs more than other US dairy herds (USDA–APHIS, 1996b; USDA–NASS, 1997). This allowed reasonable external validity and evaluation of risk factors for *Salmonella* shedding from a wide range of management practices across the country. Furthermore, fecal samples from cows in all states selected were processed in the same laboratory, thus eliminating biases due to inter-laboratory variation. Use of one laboratory, however, limited the number of samples that could be processed. This, together with the need for a large enough sample size to obtain reliable information from each herd, limited the number of herds studied. This limitation in the number of operations that could be studied resulted in an unforeseen sampling anomaly. Most of the herds in the south were sampled in the summer while most of those in the north were sampled in the spring. Another limitation was that more samples were collected from large operations than from small ones (to obtain more cull cows for another aspect of the study). Thus, it is difficult to evaluate the true effect of season and herd-size. This study, however, did identify hypotheses to be tested further (e.g., univariably there was an increase in shedding in summer compared to the spring. An increase in *Salmonella* shedding in summer compared to colder months has also been reported from the UK (Evans, 1996).

Another limitation is that in this study each cow was sampled only once to estimate point prevalence for the herd. Cattle infected with *Salmonella* shed the organism intermittently, and fecal-culture methods may underestimate the proportion of dairy herds with *Salmonella* shedders (Smith et al., 1993; Smith et al., 1994; Gay et al., 1994). Thus, we are likely to have missed some of the herds with infected cows and the herd prevalence estimate of 27.5% detected in this study is an underestimate of the true prevalence — yet a reasonable estimate of fecal shedding at any one point in time. This minimum prevalence estimate is reliable, however, because we do not expect any misclassification bias of positive operations.

In this study, an operation was classified as positive if at least one *Salmonella* isolate was recovered from the herd. Because no one serotype was found on all operations, all available isolates were used in order to increase the power to detect associations. Thus, the results of this study should be interpreted as risk factors for shedding *Salmonella* bacteria, and not for particular serotypes.

Operations where manure was removed from alleys by flushing with water had 3.5 times higher odds of having shedders than those that did not. Although with only 91 evaluated herds this association was not statistically significant (95% CI = 0.9, 14.7), this finding does agree with suggestions of Smith et al. (1993), who reported that recycling flush water on dairy operations may be a risk factor for salmonellosis in cattle. In swine barns, flushing open gutters with recycled lagoon water has also been associated with shedding of *S. agona* by pigs (Davies et al., 1997).

Operations with $\geq$100 cattle had 5.8 times higher odds of having cows shedding *Salmonella* than those with <100 cattle. Herds from the south had 5.7 times higher odds of having shedders than those in the north. Large herd-size and clustering of salmonellosis cases by region have been reported as risk factors in previous studies (Bender, 1994; Evans, 1996). The effect of large herd-size could be, in part, related to the higher tendency of large operations to bring in new cattle (USDA–APHIS, 1996b) — which risks introducing cattle that are subclinically infected with *Salmonella*. Stress as a result of transportation, overcrowding or bullying could be one of the reasons for increased risk of shedding in large operations. Furthermore, in addition to increasing the risk of transmission, other management practices are different in large herds. Thus, more-controlled studies are needed to evaluate the effects of herd-size on *Salmonella* fecal shedding.

Operations which fed brewer's products to lactating cows had 3.4 times higher odds of having *Salmonella* shedders than those which did not feed brewer's products. This association (although not significant with only 91 herds evaluated) (95% CI = 0.9, 12.9), suggests a need for further evaluation of this relationship. To the authors' knowledge, there are no previous reports associating brewer's products with *Salmonella* shedding. In this study, information on type and moisture content of brewers' products fed was not collected. Some types, e.g., wet brewers' grains have a high-moisture content (77%) (Preston, 1998), which is conducive for *Salmonella* growth. This could, in part, account for the risk associated with this product. More studies are required to further evaluate this relationship.

This was a large study with herds from 19 states across the country. The finding that an estimated 37%, 42%, 76%, or 46% cases of all operations with *Salmonella* fecal shedders could be removed by eliminating or modifying the effects of using flush water systems for manure handling, feeding brewer's products, being in a herd of more than 100 cows, or being from the southern region, respectively, is of epidemiological and clinical importance. From this analysis, it was estimated that 95% of all case herds in the dairy cow population studied could be removed by designing alternatives that reduce the effects of all four of these risk factors to their baseline levels. However, this estimate of PAR must be interpreted with caution, given that the identified risk factors may be acting through pathways that involve other factors not addressed in this study. This is likely to bias PAR upwards. However, these estimates of PAR are reasonable given that 92%, 52%, 60% and 56% of all positive operations were large herds, used flush water, fed cows brewers' products, and were from the south, respectively. The importance of the above factors is also reflected in the predicted probability of detecting a herd with a cow(s) shedding *Salmonella* given the possible various combinations of levels risk factors in the model described above. The PAR for individual risk factors do not add up to the summary PAR — indicating that these factors are not mutually exclusive (Bruzzi et al., 1985).

Although it is difficult (due to the study design) to accurately define the effects of herd-size and producers may have little control over region, this study suggests a number of management interventions for further study. For large operations, raising cattle in smaller groups on the same premises may need to be evaluated along with the economic constraints. Similarly, it might be desirable to avoid recycling flush water and to flush alleyways when cows are out of the area. Because *Salmonella* can be ubiquitous and thrives well in high-moisture environments (Smith and House, 1992), methods of manure removal from barns (gutter cleaners, alley scrapers, etc.) that do not require water may be advantageous. In the present study, univariable analysis revealed that operations which used gutter cleaners were less-likely to have *Salmonella* shedders compared to those who did not (though the latter were mainly small operations). However, after adjusting for other factors, this association became non-significant at $p < 0.05$. The potential advantages of using gutter cleaners for removing manure from alleys needs to be evaluated with a large number of herds.

From univariable analyses, the results of this study agree with those of Wray and Sojka (1977), Evans (1996), and Losinger et al. (1997) in that feeding cotton-seed products, region, and large herd-size are associated with *Salmonella* shedding. However, in the study by Anderson et al. (1997), whole cotton-seed meal was not significantly associated with a clinical disease of *Salmonella menhaden*. Animal feeds are regarded as potential risk factors for *Salmonella* shedding. In this study, feeding a TMR was significantly associated (from univariable analysis) with shedding of *Salmonella*. This result supports the findings of Anderson et al. (1997) who isolated *S. menhaden* in a TMR. However, in their study (Anderson et al., 1997), the sample of TMR was obtained from the feed bunk to which cows had access — it was not determined whether *S. menhaden* was a contaminant from an infected animal on farm or was originally in the feed. In subsequent studies, it will be necessary to culture both feces and feed ingredients for *Salmonella* isolation.

The direct effect of alfalfa on shedding of *Salmonella* is not well understood, but could be related to its use as a protein supplement instead of other protein sources. Although feeding animal fat (tallow) has been identified as a risk factor in some studies (Anderson et al., 1997; Losinger et al., 1997), we did not have sufficient power to test this factor.

Some of our findings are in contrast with reports from earlier studies. Access of other animals to dairy feeds and calving in areas also used for sick cows have been identified as significant risk factors in several studies (Smith et al., 1993; Evans, 1996; Evans and Davies, 1996). This lack of agreement could be attributed to insufficient power to detect true associations — especially where the frequency of the variable in question is small. However, our results agree with those of Evans (1996) and Losinger et al. (1997) in that application of manure on pastures or feeding some of the feedstuffs mentioned above was not associated with *Salmonella* shedding. More-targeted studies are needed to conclusively define the contribution or lack of contribution of the above factors to *Salmonella* shedding. Furthermore, a large survey is necessary to define interactions between the above factors and others not addressed in the present study.

5. Conclusion

In our present study, use of flush-water systems, feeding brewers' products, large herd-size, and region were the most important risk factors for fecal shedding of *Salmonella* in dairy cows. Furthermore, the above factors can be used to predict presence of *Salmonella* shedders in a herd. More-controlled studies are needed to understand the precise roles of each of the factors in the shedding of *Salmonella*.

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References

Altekruse, S.F., Cohen, M.L., Swerdlow, D.L., 1997. Emerging foodborne diseases. Emerging Infectious Diseases 3, 285–293.

Anderson, R.J., Walker, R.L., Hird, D.W., Blanchard, P.C., 1997. Case-control study of an outbreak of clinical disease attributable to *Salmonella menhaden* infection in eight dairy herds. J. Am. Vet. Med. Assoc. 210, 528–530.

Bender, J., 1994. Reducing the risk of *Salmonella* spread and practical control measures in dairy herds. Bovine Practitioner 28, 62–65.

Bruzzi, P., Green, S.B., Byar, D.P., Brinton, L.A., Schairer, C., 1985. Estimating the population attributable risk of multiple risk factors using case-control data. Am. J. Epidemiol. 122, 904–914.

Buntain, B., 1997. The role of the food animal veterinarian in the HACCP era. J. Am. Vet. Med. Assoc. 210, 492–495.

Buzby, J.C., Roberts, T., Jordan Lin, C.T., MacDonald, J.M., 1996. Bacterial foodborne disease: medical costs and productivity losses. USDA, Agricultural Economic Report No. 741, Washington, DC, USA.

Cullor, J.S., 1995. Common pathogens that cause foodborne disease: can they be controlled on the dairy? Vet. Med. 90, 185–194.

Davies, A., O'Neill, P., Towers, L., Cooke, M., 1996. An outbreak of *Salmonella typhimurium* DT104 food poisoning associated with eating beef. Comm. Dis. Rep. CDR. Rev. 6(11), R159–62.

Davies, P.R., Morrow, M.W.E., Jones, F.T., Deen, J., Fedorka-Cray, P.J., Gray, J.T., 1997. Risk of shedding of *Salmonella* organisms by market-age hogs in a barn with open-flush gutters. J. Am. Vet. Med. Assoc. 210, 386–389.

Evans, S.J., 1996. A case control study of multiple resistant *Salmonella typhimurium* DT 104 infection of cattle in Great Britain. Cattle Practice 4, 259–266.

Evans, S., Davies, R., 1996. Case control study of multiple resistant *Salmonella typhimurim* DT 104 infection in cattle in Great Britain. The Veterinary Record 139, 557–558.

Fedorka-Cray, P.J., Dargatz, D.A., Thomas, L.A., Gray, J.T., 1998. Survey of *Salmonella* serotypes in feedlot cattle. J. Food Prot. 61, 525–530.

Fierer, J., 1983. Invasive *Salmonella dublin* infections associated with drinking raw milk. West. J. Med. 138, 665–669.

Gay, J.M., Rice, D.H., Steiger, J.H., 1994. Prevalence of *Salmonella* shedding by cull dairy cattle in Washington State. J. Food Protection 57, 195–197.

Hosmer, D.W., Lemeshow, S., 1989. Applied Logistic Regression. Wiley, New York.

House, J.K., Smith, B.P., 1997. *Salmonella* current concepts. Proceedings of the American Association of Bovine Practitioners Meeting, No. 30, Montreal, Canada, September 1997.

Kelsey, J.L., Thompson, W.D., Evans, A.S., 1986. Methods in Observational Epidemiology. Oxford University Press, New York, pp. 254–284.

Kinde, H., Adelson, M., Ardans, A., Little, E.H., Willoughby, D., Berchtold, D., Read, D.H., Breitmeyer, R., Kerr, D., Tarbell, R., Hughes, E., 1996. Prevalence of *Salmonella* in municipal sewage treatment plant effluents in southern California. Proceedings of the 100th Annual Meeting of the United States Animal Health Association, Little Rock, Arkansas, 12–18 October 1996.

Losinger, W.C., Garber, L.P., Smith, M.A., Hurd, S.H., Biehl, L.G., Fedorka-Cray, P.J., Thomas, L.A., Ferris, K., 1997. Management and nutritional factors associated with detection of *Salmonella* sp. from cattle fecal specimens from feedlot operations in the United States. Prev. Vet. Med. 31, 231–244.

Preston, R.L., 1998. What's the feed value? Typical composition of feeds for cattle, 1998. Beef, FC2-FC15.

SAS Institute, 1994. SAS/STAT user's guide, vol. 2, GLM–VARCOMP. SAS institute, Inc., Cary, NC, USA.

SAS Institute, 1995. Logistic regression examples using the SAS system, version 6, 1st ed. SAS Institute, Cary, NC, USA, pp. 87–92.

Smith, B.P., House, J., 1992. Prospects of *Salmonella* control in cattle. Proceedings of the American Association of Bovine Practitioners (AABP) Conference, Stillwater, Oklahoma, USA, vol. 1, pp. 67–73.

Smith, B.P., Dilling, G.W., Thurmond, M.C., 1993. How common is *Salmonella* on dairies? Proceedings of the 97th Annual Meeting of the United States Animal Health Association, Las Vegas, Nevada, 23–29 October 1993.

Smith, B.P., Roden, L.D., Thurmond, M.C., Dilling, G.W., Konrad, H., Pelton, J.A., Picanso, J.A., 1994. Prevalence of Salmonellae in cattle and in the environment on California dairies. J. Am. Vet. Med. Assoc. 205, 467–471.

Smith, G.C., Morgan, J.B., Tatum, J.D., Kukay, C.C., Smith, M.T., Schnell, T.D., Hilton, G.G., 1994. The final report of the national non-fed beef quality audit. Colorado State University, Fort Collins, CO, USA.

Stokes, M.E., Davies, C.S., Koch, G.G., 1996. Categorical data analysis using the SAS system. SAS Institute, Inc., Cary, NC, USA.

USDA–APHIS, 1996a. Economic opportunities for dairy cow culling management options. USDA, APHIS, VS, CEAH, Fort Collins, CO, May, 1996.

USDA–APHIS, 1996b. Part I: Reference of 1996 dairy management practices. USDA, APHIS, VS, CEAH, NAHMS, Fort collins, Colorado, May, 1996, Publication No. N200.696.

USDA–FSIS, 1996. United States Department of Agriculture, Food Safety and Inspection Service. Pathogen Reduction Hazard Analysis and Critical Control Point (HACCP) systems final rule. 9 CFR part 304, July 1996.

USDA–NASS, 1997. United States Department of Agriculture, National Agricultural Statistics Service, Washington, DC, Cattle, January 1997.

Wells, S.J., Dargartz, D.A., Ott, S.L., 1996. Factors associated with mortality to 21 days of life in dairy heifers in the United States. Prev. Vet. Med. 29, 9–19.

Werner, S.B., Humprey, G.L., Kamei, I., 1979. Association between raw milk and *Salmonella dublin* infection. Br. Med. J. 2, 238–241.

Wray, C., Davies, R.H., 1996. A veterinary view of *Salmonella* in farm animals. Public Health Lab. System Microbiol. Digest 13, 44–48.

Wray, C., Sojka, W., 1977. Reviews of the progress in dairy science: bovine salmonellosis. J. Dairy Sci. 44, 383 –425.